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Sustained release ophthalmic dexamethasone: *In vitro in vivo* correlations derived from the PK-Eye

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ABSTRACT

Corticosteroids have long been used to treat intraocular inflammation by intravitreal injection. We describe dexamethasone loaded poly-DL-lactide-co-glycolide (PLGA) microparticles that were fabricated by thermally induced phase separation (TIPS). The dexamethasone loaded microparticles were evaluated using a two-compartment, *in vitro* aqueous outflow model of the eye (PK-Eye) that estimates drug clearance time from the back of the eye *via* aqueous outflow by the anterior route. A dexamethasone dose of 0.20 ± 0.02 mg in a 50 μL volume of TIPS microparticles resulted in a clearance $t_{1/2}$ of 9.6 ± 0.3 days using simulated vitreous in the PK-Eye. Since corticosteroids can also clear through the retina, it is necessary to account for clearance through the back of the eye. Retinal permeability data, published human ocular pharmacokinetics (PK) and the PK-Eye clearance times were then used to establish *in vitro in vivo* correlations (IVIVCs) for intraocular clearance times of corticosteroid formulations. A $t_{1/2}$ of 48 h was estimated for the dexamethasone-TIPS microparticles, which is almost 9 times longer than that reported for dexamethasone suspension in humans. The prediction of human clearance times of permeable molecules from the vitreous compartment can be determined by accounting for drug retinal permeation and determining the experimental clearance *via* the anterior aqueous outflow pathway using the PK-Eye.

1. Introduction

Intravitreal (IVT) corticosteroids are routinely used in the treatment of sight threatening conditions in the back of the eye including diabetic macular edema (DME), proliferative vitreoretinopathy, endophthalmitis and uveitis. Administration by IVT injection allows the steroids to bypass the blood-retinal barrier (BRB), leading to higher drug concentrations close to the site of action in the posterior cavity (Cunningham et al., 2008). Common corticosteroids used to treat ophthalmic conditions include dexamethasone, triamcinolone acetonide (TA) and fluocinolone acetonide (FA). Unfortunately, low molecular weight, permeable molecules including corticosteroids that are dissolved in the vitreous rapidly clear from the eye displaying a relatively short $t_{1/2}$ that is of a matter of hours (i.e. 3–7 h) (Haghjou et al., 2013; Thrimawithana et al., 2011; Kwak and Amico, 1992; Laude et al., 2010). Frequent IVT injections to maintain therapeutic drug concentrations can increase the risk of serious adverse reactions including retinal detachment, endophthalmitis and vitreous hemorrhage (Lee et al., 2010).

Steroid suspensions have long been used clinically in an effort to reduce the number of IVT injections. Triamcinolone acetonide (TA) is available as a preservative-free injectable suspension for intraocular use (Triesence®, Alcon). Kenalog® is a TA injectable suspension that is indicated for intramuscular and intra-articular administration; and has been used off-label for many years (Cabrera et al., 2014; Jermak et al., 2007; Behl et al., 1976) for

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treating both anterior and posterior segment ocular diseases. To further increase the duration of action, long-acting corticosteroid implants are now clinically available (Thrimawithana et al., 2011; Choonara et al., 2010; Thakur et al., 2014; Basile et al., 2012; Anon., 2012; Basile et al., 2014; Christoforidis et al., 2012). Corticosteroids are potent, anti-inflammatory agents that are generally stable, poorly soluble and slowly dissolving and so are ideal drug substances for use in longer acting dosage forms (Cima et al., 2014). The volume of the vitreous cavity is approximately 4.2 mL, so non-sink conditions can be exploited to prolong the release kinetics of IVT implants of corticosteroids. Ozurdex® (Allergan, Inc) is a poly(lactic-co-glycolic acid) (PLGA) implant, which releases dexamethasone (0.7 mg) over a 6-month period (Medeiros et al., 2014).

Formulating a corticosteroid in a PLGA matrix allows better control of drug release compared to the dissolution of a free drug suspension. Interestingly, Ozurdex® displays a similar pharmacokinetic (PK) profile between vitrectomised and non-vitrectomised eyes (Chang-Lin et al., 2011) whereas suspensions of TA clear more quickly in vitrectomised eyes (Beer et al., 2003). However, Ozurdex® is a single depot plug, so there is limited flexibility in reducing the duration of action to less than 6-months. Thus, in an effort to exploit the controlled release properties of PLGA for IVT administered dexamethasone with the possibility of varying the dose and duration of action, we describe the preparation of dexamethasone-PLGA microparticles prepared by thermally induced phase separation (TIPS) (Ghanbar et al., 2013).

TIPS has been used to fabricate porous drug vehicles for applications in chronic wound therapy, drug delivery and tissue engineering (Foong et al., 2010). The technique used to produce TIPS microsparticles is rapid and provides high encapsulation efficiency generating spherical particles with rigid outer surfaces and longer shelf-life (Malik et al., 2016). The encapsulation yield for microparticles containing particulate infliximab was $60.4 \pm 5.9\%$ compared with $37.9 \pm 14.4\%$ for microspheres containing an emulsion of infliximab (Foong et al., 2010). Degradable polymeric microparticles can enable a prolonged therapeutic concentration to be available while the porous particle surface aids resorption and reduces the likelihood of autocatalysis associated with solid microparticles. PLGA depots are biodegradable which avoids the need for surgical removal from the eye after drug depletion (Yandrapu and Kompella, 2013). Porous PLGA microparticles are ideal for drug delivery because the amount of polymer per microparticle is reduced compared with solid microparticles of an equal size.

A two-compartment *in vitro* model of the eye, known as the PK-Eye that predicts human clearance times caused by the aqueous outflow pathway (Awwad et al., 2015) was used to determine drug release kinetics of the dexamethasone TIPS microparticles. Aqueous outflow (2.0–2.5 μL/min) is the main cause of mass transfer within the eye (Brubaker, 1982; Toris et al., 1999; Maurice, 2001; Siggers and Ethier, 2012; Ethier et al., 2004). Aqueous humor nourishes the avascular lens and cornea with outflow passing through the front of the eye. The PK-Eye provides a good estimate of human clearance times from the vitreous cavity for protein therapeutics and non-permeable low molecular weight molecules from suspension and depots (Awwad et al., 2015). Permeable low molecular weight molecules are eliminated from the vitreous cavity by both aqueous outflow and permeation through the retina *via* the retinal-choroid-sclera (RCS) pathways. Utilising the PK-Eye to estimate the clearance of low molecular weight, retinal permeable compounds such as steroids requires that we combine *in vitro* outflow clearance data from the PK-Eye, published drug permeability and *in vivo* human data, when available, to develop *in vitro in vivo* correlations (IVIVC). The goal of the work described herein was also to develop IVIVC methodology, which can be used as a surrogate for *in vivo* ocular pharmacokinetic studies during preclinical optimisation to develop sustained release ocular preparations.

2. Experimental

2.1. Materials and instrumentation

Sodium hyaluronate (1.8 MDa) was purchased from Aston Chemicals (Aylesbury, UK) and agar (solubility: 15.0 mg/mL) was obtained from Fluka Analytical (Gillingham, Dorset, UK). Visking dialysis membrane tubing (molecular weight cut off (MWCO) 12–14 kDa) was purchased from Medicell International Ltd. (London, UK). PLGA (75:25, Purasorb PDLG 7507 0.63dL/g) was obtained from Purac Biomatericals (Gorinchem, The Netherlands). Dexamethasone, dimethyl carbonate (DMC >99.9% purity) and dimethylsulphoxide (DMSO) were obtained from Sigma-Aldrich (Dorset, UK).

A 16-channel Ismatec peristaltic pump (Michael Smith Engineers Ltd., Woking, Surrey, UK) was used to generate fluid flow into the PK-Eye. For homogenisation, a T8 Ultra-Turrax homogeniser was used (Ike-Werke, Staufen, Germany). Microparticles were fabricated using a Nisco encapsulator Var D unit, fitted with a stainless steel sapphire tipped nozzle with a 150 μm orifice (Nisco Engineering, Zurich, Switzerland). Scanning electron microscopy (SEM) was achieved with a 7401-high resolution Field Emission Scanning Electron Microscope (Jeol, Tokyo, Japan). Lyophilisation was conducted using an Edwards Micro Modulyo freeze dryer (Thermo Fisher Scientific, Asheville, NC). All analyses were undertaken using an Agilent 1200 series HPLC (Agilent Technologies Inc, Santa Clara, CA, USA) equipped with Chemstation software (Agilent) and a reverse phase Synergi Polar-RP C18, 4 μm, 15 cm column (Phenomenex, Macclesfield, UK).

2.2. Preparation of the PK-Eye for in vitro studies

2.2.1. Design of the model

The design and use of the *in vitro* PK-Eye model has been previously reported (Awwad et al., 2015). Briefly, the PK-Eye is fabricated from plastic (polyacrylate) with anterior (~0.2 mL) and posterior (~4.2 mL) cavities integrated within the model. A washer with a Visking dialysis membrane (MWCO 12–14 kDa) separates the two cavities. The direction of flow and the presence of the dialysis membrane only allows solubilised drug to pass from the posterior compartment into the anterior compartment. The PK-Eye model consists of an inlet port in the posterior cavity for continuous aqueous inflow (phosphate buffered saline, PBS pH 7.4) at a rate of 2.0 μL/min and an outlet port from the anterior cavity for sample collection. An injection port is present in each cavity with a diameter of 2.0-3.0 mm to allow administration of the desired formulation into the model. The model was placed in a pre-heated oil bath at 37 °C to conduct release studies at physiological temperature.

2.2.2. Preparation of simulated vitreous

Agar (0.4 g) and hyaluronic acid (HA) (0.5 g) were each separately mixed in 100 mL of stirred hot water (Kummer et al., 2007). The agar solution was boiled to completely solubilise the agar. After boiling, the hot agar solution was mixed with HA and stirred to give a homogenous mixture to which a few drops of 0.02% sodium azide were added. The solution was left to cool for 24 h at ambient temperature (~25 °C) and formed into a gel-like consistency. The simulated vitreous was then transferred to the PK-Eye model *via* the injection port in the posterior cavity. This combination of agar and HA was found to have a dynamic viscosity